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Patent Application
Docket No. GJE-21D2
Serial No. 09/760,274

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner : Michael C. Wilson
Art Unit : 1632
Applicant : John Sinden, Jeffrey A. Gray, Helen Hodges, Timothy Kershaw,
Fiza Rashid-Doubell
Serial No. : 09/760,274
Filed : January 12, 2001
For : Neural Transplantation Using Pluripotent Neuroepithelial Cells

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF JOHN SINDEN, Ph.D., UNDER 37 C.F.R. § 1.132

Sir:

I, John Sinden, Ph.D., of ReNeuron Limited, hereby declare:

THAT, my *curriculum vitae* is attached hereto as Exhibit A;

THAT, I am a named inventor on the above-referenced patent application;

THAT, I have read and understood the specification and claims of the subject application and the Office Action dated May 31, 2002;

AND, being thus duly qualified, do further declare:

1. Our invention is based on the surprising discovery that conditionally immortalized, nestin-positive, pluripotent neuroepithelial cells, when transplanted into a damaged brain, are capable of differentiating into the phenotype of the damaged part of the brain, thereby repairing the damage and restoring function that has been lost as a result of the damage. Furthermore, it has been found that these cells have the property of migrating from the site of application to the site of damage. Further work (for which evidence is now provided) confirms these initial observations, and provides evidence that a wide variety of human brain damage-related disorders are capable of treatment by using nestin-positive, pluripotent neuroepithelial cells that have been genetically modified to be conditionally immortal.

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2. Previously, it was thought that to treat damage in a developed postnatal or adult brain, it was necessary to use tissue/cells derived from the same area as that damaged. Importantly, prior to our invention, even if the cells to be transplanted were taken from a fetus, such as those described in the Netto *et al.* publication, the cells would typically be committed to a particular phenotype. Moreover, prior to our work, there was no selection of nestin-positive, pluripotent cells, or genetic modification of the cells to confer conditionally immortality such that the cells would be immortal prior to transplantation but differentiate subsequent to transplantation. We have realized that, surprisingly, transplanting cells that were selected to retain a nestin-positive, pluripotent, conditionally immortal phenotype resulted in the repair of damage, and this was independent of the site of damage.

3. In contrast to the observations made in the Scheffler *et al.* publication (*Brain and Bone Marrow*, 1999, 11:348-357), which is cited by the Reviewer in the Office Action, one of the great advantages of the present invention is that it is not necessary to target particular areas of the brain to correct cell damage. The cells used in our invention migrate to areas of damage after transplantation and become integrated in the damaged areas, effecting repair. This ability of the cells to migrate (which we were the first observe) is an inherent feature of the cells; therefore, the difficulties identified in the Scheffler *et al.* publication will not be experienced when using nestin-positive, pluripotent neuroepithelial cells that have been genetically modified to be conditionally immortal.

4. The Reviewer cites the Sinden *et al.* (1997) publication as suggesting that hippocampal CA1 cells must be used to repair CA1 tissue. However, the statement referred to by the Reviewer within the Sinden *et al.* publication (of which I am the first author), is made with respect to a previous study that used primary cells that were mature, differentiated or committed CA1 cells, and not the conditionally immortal, pluripotent, nestin-positive neuroepithelial cells that are used in the method of our invention. Provided the neuroepithelial cells are nestin-positive and retain the ability to differentiate into the specified phenotypes in response to environmental signals, they are appropriate

for use in the present invention. Nestin-positive cells can be readily identified using immunocytochemistry, for example.

5. The ability of conditionally immortal nestin-positive, pluripotent neuroepithelial cells to migrate is readily apparent in U.S. Patent Application Publication No. 2002/0037277 (hereinafter the '277 publication), which is attached as Exhibit B. The example at pages 2-5 of the '277 publication describes implantation of conditionally immortal, nestin-positive, pluripotent neuroepithelial cells into the unilaterally damaged brains of rats. The cells were implanted either ipsilaterally or contralaterally to the hemisphere containing the infarcted area, resulting in improved function. Compelling evidence of extensive migration is presented at page 4, paragraph 0047, which indicates that contralaterally grafted cells "migrated across the midline to the opposite side of the brain" (emphasis added). Certainly, the experiment shows that cells from one anatomical region of the brain (hippocampal region) can repair damage to a different anatomical region of the brain, such as cortex and basal ganglia.

6. The Reviewer refers to Sanberg *et al.* as teaching that immunosuppressive agents used for xenotransplanted cells may preclude any therapeutic benefit in humans because of the health risks associated with immunosuppression. However, in all forms of transplantation therapy, *e.g.*, liver, heart, *etc.*, immuno-suppressive agents are virtually always included as part of the treatment regimen to help avoid rejection. Also submitted herewith as Exhibit C is the Virley *et al.* publication (*Brain*, 1999, 122:101-115), which supports the efficacy of transplanting conditionally immortal nestin-positive, pluripotent cells into the damaged mammalian brain to restore function caused by cell damage or cell loss. The Virley *et al.* publication, of which I am a co-author, describes the implantation of pluripotent MHP36 cells into the bilaterally lesioned brains of primates (marmosets), resulting in improved function. These MHP36 cells, which are mouse cells, are also exemplified in our patent application. These cells performed as well within the marmoset brain as the marmoset fetal allografts, which suggests a low immune response provocation and confirms the applicability of

our invention to treat primates, in general, including humans, with a reasonable expectation of success.

7. The Morris water maze test was developed in the 1980s and has become the method of choice for assessment of spatial learning and memory in rodents. The test is also accepted by those skilled in the art as generally predictive of long-term neurological and behavioral outcome in humans and is routinely utilized in the development of repair strategies for human neuro-degenerative disorders. This is evidenced by the enormous number of publications in the field in which this model is utilized. A survey of the literature shows a general consensus that the water maze test is useful in assessing cognitive function (e.g., spatial learning and memory) within the context of a variety of etiologies (e.g., aging, neonatal stress, lesion or ischemic damage to cortex, striatum, or hippocampus) and treatments (Morris R.G.M. "Spatial localisation does not require the presence of local cues" *Learn Motiv.*, 12: 239-260, 1981; Rapp. P.R. *et al.* "An evaluation of spatial information processing in aged rats" *Behav. Neuroscience*, 101:3-12, 1987; Di Mattia R. and Kesner P. "Spatial cognitive maps: differential role of parietal cortex and hippocampal formation" *Behav. Neuroscience*, 102:471-480, 1988; Stewart C.A. *et al.* The water maze. In *Behavioural Neuroscience: a practical approach*, Rickwood D and Hames H.D. (eds), Oxford. OUP, pp106-122, 1993; Propoli P. *et al.* Behavioural and electrophysiological correlates of the quinolinic acid lesion model of Huntington's Disease in rats, 1994; Hodges H. Testing for spatial brain dysfunction in animals. In: *Handbook of Spatial Learning*, eds N. Foreman and R. Gillett, Chapter 15, 1998; McIlwain, Merriweather M.Y. *et al.* "The use of behavioural test batteries: effects of training history" *Physiol and Behav.*, 73:705-17, 2001).

8. The Wisconsin General Test Apparatus has been consistently used world wide for testing cognitive function in primates at least since the 1940s, across a range of species including Rhesus, Cynomolgus, and Squirrel monkeys, and marmosets, as demonstrated by the Harlow publication (Harlow, H.F., "A test apparatus for monkeys", *Psychological Record*, 2, 434-436, 1938), and

subsequent publications (Harlow, H.F., "The formation of learning sets", *Psychol. Rev.*, 56:51-65, 1949; Ridley, R.M. *et al.*, "A new approach to the role of noradrenaline in learning: problem-solving in the marmoset after alpha-noradrenergic receptor blockade", June, 14(6):849-855, 1981; Ridley R.M. *et al.* "Cholinergic learning deficits in the marmosets produced by scopolamine and ICV hemicholinium" *Psychopharmacology* 83, 340-345, 1984; Roberts, A.C. "Comparison of cognitive function in human and non-human primates" *Cognitive Brain Res.* 3, 319-327, 1996; and Ridley R.M., and Baker HF "Evidence for specific information processing in monkeys with lesions of the septohippocampal system" *Cortex* 33, 167-76, 1997).

9. A human pluripotent clonal cell line (designated "CX") has now been developed as well. As described in Exhibit D, which is attached hereto, these cells were conditionally immortalized using the same techniques as described in the patent application. Following confirmation of their clonality, the human conditionally immortal, pluripotent cells were implanted into the brains of rats having unilateral basal forebrain excitotoxic lesions, which mimics some of the cell loss that occurs in Alzheimer's disease and other neurodegenerative conditions. As described in Exhibit D, effects of the human cell line were assessed in comparison with several murine cell lines including the MHP36 cell line as a positive control, and with sham-grafted lesioned and non-lesioned controls. As expected, rats grafted with the murine MHP36 cell line performed significantly better than lesioned animals. However, rats receiving the human cell line showed as rapid spatial learning as controls, and were superior both to the lesion and the murine grafted groups relative to lesion-only animals in a memory task.

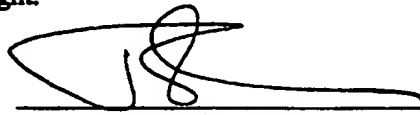
10. The Reviewer cites the Netto *et al.* publication as describing the method of our invention. However, as described above, the Netto *et al.* publication, describes the transplantation of CA1 cells from late fetal stages, *e.g.*, embryonic day (E) 19-20; these cells are typically mature, differentiated cells and hence not pluripotent or nestin-positive. Furthermore, the cells have not been genetically modified to confer conditional immortality.

11. Based on the ability of conditionally immortal pluripotent, nestin-positive neuroepithelial cells to migrate to, and adopt the phenotype of, any damaged part of the brain, and based on the experimental data showing restored function in a variety of brain damage models, there is no reason to doubt that the invention will be applicable in treating a variety of conditions, including those characterized by cognitive deficits. Furthermore, based on the teaching of the specification and further supported by the experimental data, there is no reason to doubt that restoration of cognitive function can be achieved in a variety of mammals, including humans.

The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or of any patent issuing thereon.

Further declarant sayeth naught.

Signed:


John Sinden, Ph.D.

Date:

27th September 2002